Zootaxa 1944: 34–52 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Descriptions of two new species of Hemileucinae (Lepidoptera: Saturniidae) from the region of Muzo in Colombia—evidence from morphology and DNA barcodes

THIBAUD DECAËNS[1, 2] & RODOLPHE ROUGERIE[2, 3]

[1]*Laboratoire d'Écologie, ECODIV – EA 1293, Federation de Recherche SCALE, U.F.R. Sciences et Techniques, Université de Rouen, F–76821 Mont Saint Aignan cedex, France. E-mail: thibaud.decaens@univ-rouen.fr*
[2]*Muséum National d'Histoire Naturelle, Département Systématique et Evolution, USM 602, case postale n°50 (Entomologie), F-75231 Paris cedex 05*
[3]*Biodiversity Institute of Ontario, University of Guelph, 579 Gordon Street, N1G 2W1, Guelph, Ontario, Canada. E-mail: rrougeri@uoguelph.ca*

Abstract

Two new species of Hemileucinae are described from the region of Muzo (Boyaca department) in the Eastern Cordillera of Colombia. *Leucanella bonillensis*, **new species**, is a small greyish species whose closest relatives are *L. newmani* (Lemaire) and *L. acutissima* (Walker). It can be distinguished from those two species by several subtle differences in wing pattern and coloration as well as a few characters of the male genitalia, which are overall very conserved within the genus. *Cerodirphia zulemae*, **new species**, belongs to the very uniform species-group of *C. speciosa* (Cramer), characterised by a pink ground colour and the presence of a "Y"-shaped discal mark on the forewing. Based on its male genitalia, the new species is related to *C. brunnea* (Draudt) and *C. apunctata* Dias & Lemaire. It may be distinguished from the former by its more vivid ground colour, but detailed examination of the male genitalia are necessary to differentiate it from *C. apunctata*. Colour pictures of the habitus of the new species and their relatives are provided, and their genital structures are figured as well, including both sexes for *C. zulemae*. We also provide additional support to these descriptions based on genetic data obtained in the context of a global DNA barcoding campaign recently initiated for saturniid moths. Both *L. bonillensis* and *C. zulemae* are unambiguously distinguished from closest relatives based on genetic distances (no intraspecific distances in either case; interspecific distance ranges 5.6–6.6% and 6.7–12.5%, respectively) and inference of phylogenetic hypotheses based on partial sequences of the COI mitochondrial gene. These results emphasize the potential of DNA barcoding to support taxonomic work in species-groups considered difficult to address through morphology.

Key words: Eastern Colombia, *Leucanella bonillensis* **n. sp.**, *Cerodirphia zulemae* **n. sp.**, Neotropical insects, cryptic species, DNA barcoding, COI

Résumé

Deux nouvelles espèces d'Hemileucinae sont décrites de la région de Muzo (département du Boyaca) dans la Cordillère Orientale de Colombie. *Leucanella bonillensis* **nov. sp.** est une espèce grisâtre de taille modeste, proche de *L. newmani* et de *L. acutissima*. Elle peut être distinguée superficiellement de *L. newmani* et *L. acutissima* par des différences subtiles dans l'ornementation et la couleur des ailes, ainsi que quelques caractères sur les genitalia mâles qui sont cependant très conservés au sein du genre. *Cerodirphia zulemae* nov. sp. est une espèce typique du groupe de *C. speciosa*, qui se caractérise par la coloration rose des ailes et la présence d'une structure discocellulaire en forme de "Y" sur les ailes antérieures. De part la structure des genitalia, elle se rapproche de *C. brunnea* et *C. apunctata*. Elle peut être différenciée de la première par sa coloration générale plus vive, mais une observation détaillée des genitalia est nécessaire pour la

 Accepted by J. W. Brown: 1 Oct. 2008; published: 28 Nov. 2008

séparer de *C. apunctata*. La distinction de ces deux nouveaux taxons est confirmée par l'analyse de données moléculaires obtenues dans le cadre d'une campagne globale de DNA barcoding initiée récemment et visant la famille des Saturniidae. La comparaison des distances génétiques intra- et interspécifique (distances intraspécifiques nulles dans les deux cas; distances interspécifiques de 5.6–6.6 et 6.7–12.5 respectivement pour *L. bonillensis* et *C. zulemae*), ainsi que l'analyse phylogénétique des séquences partielles du gène mitochondrial COI permettent de séparer sans ambiguïté *L. bonillensis* et *C. zulemae* des especes les plus proches; ces résultats démontrent le potentiel du code barre ADN comme support taxonomique dans les groupes d'espèces considérés comme difficiles à étudier sur la base de leur morphologie.

Mots clefs: Colombie Orientale, *Leucanella bonillensis* **n. sp.**, *Cerodirphia zulemae* **n. sp.**, insectes néotropicaux, espèces cryptiques, code barre ADN, COI

Resumen

Dos nuevas especies de Hemileucinae se describen para la región de Muzo (departamento de Boyacá) en la Cordillera Oriental de Colombia. *Leucanella bonillensis* **nov. sp.** es una especie gris de tamaño mediano, cercana a *L. newmani* y *L. acutissima*. Se puede distinguir superficialmente de *L. newmani* y *L. acutissima* por diferencias sutiles en los patrones y el color de las alas, así como en los caracteres de los genitalia de los machos. *Cerodirphia zulemae* **nov. sp.** es una especie característica del grupo de *C. speciosa*, caracterizado por la coloración rosada de las alas y la presencia de una estructura discocelular en forma de "Y" en las alas anteriores. Según la estructura de los genitalia, se acerca a *C. brunnea* y *C. apunctata*. Se puede diferenciar de la primera por la coloración mas viva de las alas, pero es necesaria una observación detallada de los genitalia para separarla de *C. apunctata*. El análisis de los datos moleculares obtenidos a partir de una campaña global de códigos de barras de ADN, iniciada recientemente con la familia Saturniidae, confirma la distinción de estas nuevas especies. La comparación de las distancias genéticas intra- e interespecíficas (distancias intraespecíficas de 0% para ambas espécies; distancias interespecíficas de 5.6–6.6 y 6.7–12.5 respectivamente para *L. bonillensis* y *C. zulemae*), así como el análisis filogenético de las secuencias parciales del gen mitocondrial COI, permiten separar sin ambigüedad *L. bonillensis* y *C. zulemae* de las especies cercanas. Estos resultados demuestran el potencial de los códigos de barras como ayuda taxonómica para aquellas especies que son difíciles de estudiar a través de la morfología.

Palabras claves: Colombia Oriental, Saturniidae, *Leucanella bonillensis* **n. sp.**, *Cerodirphia zulemae* **n. sp.**, taxonomía, insectos neotropicales, especies crípticas, códigos de barras de ADN, COI

Introduction

Saturniidae is a group of generally large-bodied moths that comprises about 1,600 described species distributed worldwide. In the Americas, as many as 921 described species were cited by Lemaire (1996) in a comprehensive checklist of the family, but that number has increased dramatically over the last decade. The highest recorded diversity and endemism are in the Andean region, with about 35% of the total number of species and as many as 200 endemics (Lemaire 1978; Amarillo-Suarez 2000). Located at the intersection of various biogeographical regions, Colombia probably hosts one of the most diverse saturniid faunas of Tropical America (Amarillo-Suarez 2000; Decaëns *et al.* 2003c), although saturniids have received little attention compared to some groups of butterflies and beetles (Coleoptera). A checklist of the Saturniidae of Colombia published by Amarillo-Suarez (2000) includes 185 species of the subfamilies Saturniinae, Arsenurinae, Hemileucinae, and Ceratocampinae; it excludes Cercophaninae and Oxyteninae, two small subfamilies treated as distinct families in the past but now included in Saturniidae on the basis of morphological and molecular analyses (Minet 1994; Regier *et al.* 2008). This number accounts for about 20% of the total number of American species, but is probably an underestimate; civil unrest has discouraged intensive insect collecting in Colombia during the past 50 years. This fact was recently reinforced by the results of new collecting efforts, which resulted in the re-discovery of species not observed since the 1940s (Amarillo & Wolfe 1996; Wolfe *et al.* 2003a) and the discovery of several new species (Lemaire & Amarillo 1992; Decaëns 2003; Decaëns *et al.*

2003a, 2003b, 2003d), plus provided key specimens for the revision of misunderstood species groups (Wolfe *et al.* 2003b).

In December 2002, night collecting was conducted by D. Bonilla and G. Lecourt at 1500 m elevation in an Andean forest in the region of Muzo (Boyaca, Colombia). A second collecting trip was made 14–18 April 2003 in the same region, but at slightly higher elevation. Laboratory study of the material revealed the presence of several interesting species, including the poorly known *Copaxa apollinairei* Lemaire (Decaëns et al. 2007) and two previously undescribed members of the subfamily Hemileucinae in the genera *Cerodirphia* and *Leucanella*, whose formal descriptions are given in this paper. The taxonomy of these two genera is difficult owing to the very strong homogeneity of adult phenotypes: (1) in *Cerodirphia*, the wing pattern of members of the *C. speciosa* species-group, to which the specimens studied in this paper belong, is very conserved, providing almost no reliable diagnostic characters; (2) in *Leucanella* most of the variation is manifested in the shape, size, and structure of the hindwing eye-spot, and this variation often is difficult to interpret. Moreover, the genital morphology in the latter genus provides little evidence for distinguishing closely related species. In order to address these pitfalls and provide additional evidence in support of our hypotheses of the newly delineated species, we analyzed partial COI sequences obtained in the context of the global DNA barcoding campaign for saturniid moths (see www.lepbarcoding.org). This portion of the mitochondrial genome was proposed by Hebert et al. (2003) as a DNA barcode - a molecular identifier of animal species.

Materials and methods

Specimen collecting and morphological study

Collecting was conducted in the following localities: (1) 1–3 December 2002 in Municipio de Quipama, Vereda Caviche, 1500 m a.s.l. (G. Lecourt and D. Bonilla leg.); (2) 14–18April 2003 in Municipio de Arcabuco, 2000 m a.s.l. (D. Bonilla and L. D. Ramirez leg.). The vegetation at both sites is humid Andean forest with a moderate level of fragmentation. Moths were attracted by a 175W mercury vapour bulb powered by a small portable generator. A white sheet 2 m high × 3 m wide was used as a reflector. Trapping was done throughout each entire night, i.e. from 1830 to 0630 hrs. Moths were collected as soon as they arrived at the sheet, injected with ammonia, and stored and dried in labelled paper envelopes. Collected specimens were brought to the lab, relaxed in a humid box, mounted to allow observation of wing patterns, and deposited in the collections of the authors. Identifications followed Lemaire (1978, 1980, 1988, 2002) and Jordan (1924) (see Decaëns et al. 2007 for a complete list of the species collected in these two localities). Two presumed undescribed taxa in the genera *Leucanella* and *Cerodirphia* were isolated from the rest of the collection for further study and description. Genital parts (male and female when possible) were prepared in a standard way using 10% caustic potash solution.

For the sake of comparison, we examined specimens of the following related species: *Leucanella acutissima* (Walker) (10 male specimens), *L. memusae gardineri* (Lemaire) (5 male specimens), *L. newmani* (Lemaire) (7 male specimens), *L. viridescens viridior* (Lemaire) (9 male specimens); *Cerodirphia apunctata* Dias & Lemaire (5 male specimens), *C. brunnea* (Draudt) (5 male specimens), *C. speciosa* (Cramer) (11 male and 2 female specimens). These specimens are deposited in the collections of the authors, the Muséum National d'Histoire Naturelle in Paris, and the research collection of Daniel Herbin (France, Toulouse).

We used the revision of the subfamily Hemileucinae by Lemaire (2002) as a source of additional data for comparison of morphological features and geographic ranges among the new species and their relatives. We also used this work to verify that the new species have not been described previously and/or synonymised with similar taxa.

DNA barcoding analysis

DNA was extracted from dry legs removed from mounted collection specimens. We sampled four specimens of the presumed new *Cerodirphia* species and two of the presumed new *Leucanella* species, and we included in the analyses several sequences of closely related species obtained for the global DNA barcoding campaign targeting saturniid moths and maintained in the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007). Table 1 gives a summary of the specimens used for the genetic study, with details on their geographic origin, the location of the vouchers, and the GenBank sequence accession numbers. We used *C. opis* (Schaus), *C. avenata* (Draudt), and *L. stuarti* (Rothschild & Jordan) as outgroups for the phylogenetic analyses. Data, images, and sequences for non-type specimens are publicly available in the following projects 'Saturniidae – Decaëns collection PUBLIC records' (code TDPUB), 'Saturniidae – Rougerie collection PUBLIC records' (code RRPUB), and 'Saturniidae – Herbin collection PUBLIC records' (code DHPUB) within the Published Projects section of BOLD. Type specimens are grouped, in this same section of BOLD, in a different project named 'Saturniidae - Type specimens'.

Tissue samples were processed at the Canadian Centre for DNA Barcoding (CCDB). DNA was extracted from dry legs using a routine silica-based 96-well extraction automation protocol (Ivanova *et al.* 2006). The part of CO1 used as a 'DNA barcode' (Hebert et al. 2003) was amplified with the primer set LepF1/LepR1 (Hebert *et al.* 2004), targeting a 658bp fragment. The DNA extracts that did not amplify for the full-length DNA barcode were hitpicked and re-amplified with the LepF1/EnhLepR1 (Hajibabaei *et al.* 2006) primer pair, targeting a 612-bp fragment of COI, and the subsequently remaining failures went through a third amplification attempt using the internal primer pairs LepF1/MLepR1 and MLepF1/LepR1, targeting respectively DNA fragments of 307bp and 405bp (Hajibabaei *et al.* 2006). All PCR amplifications were performed according to the standard PCR reaction protocol used in CCDB (Hajibabaei *et al.* 2005); PCR products were checked on a 2% E-gel® 96 Agarose (Invitrogen, Burlington, ON, Canada). Unpurified PCR fragments were sequenced in both directions using the same primers as for the PCR reaction. The sequencing reactions followed CCDB protocols (Hajibabaei *et al.* 2005), with products subsequently purified using Agencourt® CleanSEQ protocol (Agencourt, Beverly, MA, USA). The sequences were managed in SeqScape version 2.1.1 (Applied Biosystems, Foster City, CA, USA) or Sequencher 4.5 (Gene Code Corporation, Ann Arbor, MI, USA) and aligned using bioedit version 7.0.5.3 (Hall 1999) or MEGA4 (Tamura *et al.* 2007). Regularly updated protocols used at the CCDB can be found at the following URL: http://www.dnabarcoding.ca/pa/ge/research/protocols.

Phylogenetic analysis of DNA sequences and genetic distances calculation

The sequences were downloaded from BOLD and analyzed using NONA 2.0 (Goloboff 1999) in Winclada 1.00.08 (Nixon 2002) for a cladistic analysis. A heuristic search was performed using a branch swapping algorithm with the following parameters: hold1000000, mult*500, hold/100 and max*. Bootstrap values were used to estimate branch support; they were calculated in Winclada after 1000 random replications using the same set of parameters as given above. Distance calculations were performed using the Kimura 2-parameter (K2P) method in MEGA4 (Kimura 1980; Tamura *et al.* 2007) including all sites, with the pairwise deletion option and assuming both an homogeneous pattern of divergence among lineages and a uniform rate of substitutions among sites.

Abbreviations used

TD = Thibaud Decaëns, RR = Rodolphe Rougerie, DH = Daniel Herbin, MNHN = Muséum national d'Histoire naturelle of Paris (France), INCN = Instituto Nacional de Ciencias Naturales de Santafé de Bogota (Colombia).

TABLE 1. Details of the 53 specimens used for the genetic analysis. L=sequence length; Dep.=depository collection (TD = Thibaud Decaëns collection; RR = Rodolphe Rougerie collection; MNHN = Muséum National d'Histoire Naturelle, Paris; INCN = Instituto Nacional de Ciencias Naturales, Bogota; DH = Daniel Herbin collection); F=female; M=male. SampleID and ProcessID are unique identifiers; they refer, respectively, to the voucher specimen and sequence information on BOLD. Holotypes of the two species described are highlighted in bold characters, as well as the paratype of *Cerodirphia apunctata*.

Sample ID	Process ID	L	Identification	GenBank #	Sex	Dep.	Date Coll.	Country, province	Alt. (m)
BC-Her0253	SDHA253-07	648	*Leucanella acutissima*	FJ026985	F	DH	27-Aug-1996	Mexico, Oaxaca	600
BC-Her0252	SDHA252-07	609	*Leucanella acutissima*	FJ026986	M	DH	31-juil-92	Mexico, Oaxaca	1250
BC-Her1291	SDHB291-07	658	*Leucanella acutissima*	FJ026989	M	DH	17-May-2007	Guatemala, Izabal	1190
BC-Her1278	SDHB278-07	658	*Leucanella acutissima*	FJ026990	F	DH	09-May-2007	Guatemala, Suchitepequez	1450
BC-Her1277	SDHB277-07	658	*Leucanella acutissima*	FJ026991	M	DH	07-May-2007	Guatemala, Suchitepequez	1041
BC-Her1292	SDHB292-07	658	*Leucanella acutissima*	FJ026988	M	DH	07-May-2007	Guatemala, Suchitepequez	1041
BC-Her1363	SDHB363-07	658	*Leucanella acutissima*	FJ026987	F	DH	08-May-2007	Guatemala, Suchitepequez	1420
BC-Her1397	SDHB397-07	658	*Leucanella acutissima*	FJ026993	M	DH	07-May-2007	Guatemala, Suchitepequez	1041
BC-Her1399	SDHB399-07	658	*Leucanella acutissima*	FJ026992	M	DH	09-May-2007	Guatemala, Suchitepequez	1450
BC-Dec0006	SATWA038-06	658	*Leucanella bonillensis* n. sp.	FJ026994	M	TD	01-Dec-2003	Colombia, Boyaca	1500
BC-Dec0005	**SATWA037-06**	**658**	***Leucanella bonillensis* n. sp.**	FJ026995	**M**	**INCN**	**01-Dec-2002**	**Colombia, Boyaca**	**1500**
BC-Her0296	SDHA296-07	658	*Leucanella memusae gardineri*	FJ027000	M	DH	16-oct-04	Brazil, Minas Gerais	700
BC-Her0648	SDHA648-07	658	*Leucanella memusae gardineri*	FJ026996	M	DH	12-nov-04	Brazil, Minas Gerais	700
BC-Her0299	SDHA299-07	609	*Leucanella memusae gardineri*	FJ026997	M	DH	30-Aug-2000	Brazil, Espirito Santo	600
BC-Her0298	SDHA298-07	564	*Leucanella memusae gardineri*	FJ026998	M	DH	30-Aug-2000	Brazil, Espirito Santo	600
BC-Her0297	SDHA297-07	658	*Leucanella memusae gardineri*	FJ026999	M	DH	16-oct-04	Brazil, Minas Gerais	-
BC-Her0278	SDHA278-07	504	*Leucanella newmani*	FJ027004	M	DH	26-juil-94	Bolivia, Beni	350
BC-Her0223	SDHA223-07	658	*Leucanella newmani*	FJ027006	F	DH	26-oct-00	Bolivia, Santa Cruz	860
BC-Her0276	SDHA276-07	609	*Leucanella newmani*	FJ027005	M	DH	27-juil-94	Bolivia, Beni	250
BC-Dec0533	STDA523-07	658	*Leucanella newmani*	FJ027001	M	TD	16-nov-91	Bolivia, La Paz	310
BC-Dec0004	SATWA036-06	658	*Leucanella newmani*	FJ027002	M	TD	01-oct-93	Bolivia, La Paz	310
BC-Her0279	SDHA279-07	609	*Leucanella newmani*	FJ027003	M	DH	06-Aug-1994	Bolivia, Beni	400
BC-Her0237	SDHA237-07	658	*Leucanella stuarti*	FJ027007	M	DH	25-oct-00	Bolivia, Chuquisaca	2360
BC-Roug0212	SATWA231-07	658	*Leucanella viridescens viridior*	FJ027009	F	RR	01-janv-06	Brazil, Sao Paulo	460
BC-Dec0530	STDA520-07	658	*Leucanella viridescens viridior*	FJ027008	F	TD	24-Dec-1997	Bolivia, La Paz	2200
BC-Her0222	SDHA222-07	658	*Leucanella viridescens viridior*	FJ027010	M	DH	13-nov-98	Bolivia, Chuquisaca	1650

TABLE 1. (continued)

Sample ID	Process ID	L	Identification	GenBank #	Sex	Dep.	Date Coll.	Country, province	Alt. (m)
BC-Her0221	SDHA221-07	658	*Leucanella viridescens viridior*	FJ027011	M	DH	13-nov-98	Bolivia, Chuquisaca	1650
BC-Her0220	SDHA220-07	658	*Leucanella viridescens viridior*	FJ027012	M	DH	13-nov-98	Bolivia, Chuquisaca	1650
BC-Her0219	SDHA219-07	658	*Leucanella viridescens viridior*	FJ027013	M	DH	13-nov-98	Bolivia, Chuquisaca	1650
BC-MNHN0005	SATWA049-06	573	*Cerodirphia apunctata*	FJ026962	M	MNHN	20-nov-90	Venezuela, Bolivar	1400
BC-MNHN0004	**SATWA048-06**	**383**	***Cerodirphia apunctata***	**FJ026961**	**M**	**MNHN**	**07-Feb-1985**	**Brazil, Sao Paulo**	**820**
BC-Dec1082	STDB132-07	658	*Cerodirphia avenata*	FJ026963	M	TD	01-Aug-2005	Costa Rica, Cartago	1560
BC-Her1022	SDHB022-07	652	*Cerodirphia brunnea*	FJ026965	M	DH	23-nov-03	Argentina, Jujuy	1680
BC-Dec0007	SATWA039-06	573	*Cerodirphia brunnea*	FJ026964	M	TD	01-nov-90	Bolivia, La Paz	1450
BC-Her0175	SDHA175-07	658	*Cerodirphia brunnea*	FJ026966	M	DH	28-oct-00	Bolivia, Chuquisaca	1245
BC-Her0174	SDHA174-07	636	*Cerodirphia brunnea*	FJ026967	M	DH	28-oct-00	Bolivia, Chuquisaca	1245
BC-Roug0550	SATWA569-07	658	*Cerodirphia opis*	FJ026968	M	RR	01-Apr-2005	Brazil, Santa Catarina	890
BC-Dec0496	STDA486-07	623	*Cerodirphia speciosa*	FJ026973	M	TD	01-nov-91	Bolivia, La Paz	310
BC-Her0302	SDHA302-07	609	*Cerodirphia speciosa*	FJ026979	M	DH	04-Aug-1988	Ecuador, Morona-Santiago	800
BC-Roug0030	SATWA043-06	646	*Cerodirphia speciosa*	FJ026974	M	RR	29-sept-03	Peru, Pasco	600
BC-Roug0029	SATWA042-06	658	*Cerodirphia speciosa*	FJ026975	M	RR	29-sept-03	Peru, Pasco	600
BC-Roug0028	SATWA041-06	573	*Cerodirphia speciosa*	FJ026976	F	RR	01-nov-92	Bolivia, La Paz	270
BC-Her0308	SDHA308-07	658	*Cerodirphia speciosa*	FJ026980	M	DH	25-nov-98	Bolivia, Beni	850
BC-Her0307	SDHA307-07	658	*Cerodirphia speciosa*	FJ026977	M	DH	28-juil-94	Bolivia, Beni	400
BC-Dec0500	STDA490-07	658	*Cerodirphia speciosa*	FJ026969	M	TD	01-nov-91	Bolivia, La Paz	310
BC-Dec0499	STDA489-07	658	*Cerodirphia speciosa*	FJ026970	F	TD	01-nov-91	Bolivia, Beni	310
BC-Dec0498	STDA488-07	621	*Cerodirphia speciosa*	FJ026971	F	TD	01-nov-91	Bolivia, Beni	310
BC-Dec0497	STDA487-07	576	*Cerodirphia speciosa*	FJ026972	M	TD	01-nov-91	Bolivia, Beni	310
BC-Her0303	SDHA303-07	658	*Cerodirphia speciosa*	FJ026978	M	DH	04-Aug-1988	Ecuador, Morona-Santiago	800
BC-Dec0491	STDA481-07	658	*Cerodirphia zulemae* n. sp.	FJ026981	M	TD	01-Dec-2002	Colombia, Boyaca	1500
BC-Dec0010	**SATWA047-06**	**658**	**Cerodirphia zulemae n. sp.**	**FJ026982**	**M**	**INCN**	**01-Dec-2002**	**Colombia, Boyaca**	**1500**
BC-Dec0009	SATWA046-06	658	*Cerodirphia zulemae* n. sp.	FJ026983	M	TD	01-Dec-2002	Colombia, Boyaca	1500
BC-Dec0008	SATWA045-06	621	*Cerodirphia zulemae* n. sp.	FJ026984	M	TD	01-Dec-2002	Colombia, Boyaca	1500

Results and species descriptions

The comparative morphological study confirmed the existence of a number of diagnostic characters allowing distinction of two new species of *Leucanella* and *Cerodirphia* within the specimens collected in the region of Muzo. In the former genus, these characters mostly involve features of the wing pattern, whereas in the second genus, genital comparison is the only effective way to properly discriminate the new species (see description and diagnosis below).

We obtained DNA barcode sequences from all 6 specimens we processed for these two new species, and included them in datasets gathering sequences of closely related taxa (Table 1). The *Leucanella* dataset includes 29 sequences: 2 sequences of the newly described species, 9 sequences for *L. acutissima*, 6 for *L. viridescens viridior*, 5 for *L. memusae gardineri*, 6 for *L. newmani*, and 1 for *L. stuarti* as the outgroup used to root the tree. The *Cerodirphia* dataset includes 24 sequences: 4 sequences for the newly described species, 2 sequences for *C. apunctata* (including a paratype of this species), 4 sequences for *C. brunnea*, 12 sequences for *C. speciosa*, and unique sequences of *C. opis* and *C. avenata* as outgroup taxa. Most of the sequences used in the analysis of the complete dataset were full-length DNA barcodes (658bp), and we accommodated the few shorter ones (see Table 1) by treating alignment gaps as missing data in Winclada. The genetic datasets for the genera *Leucanella* and *Cerodirphia*, analyzed independently, encompass respectively 69 and 111 (96 within ingroup) informative characters.

The phylogenetic analysis of the *Leucanella* dataset resulted in a single tree (Fig. 1), whereas nine equally parsimonious trees were obtained for *Cerodirphia*, the strict consensus of which is shown in Fig. 2. All of the currently recognized taxa in the analyses are reciprocally monophyletic and all of the groupings relevant to the distinction of the two new Colombian species are strongly supported by the molecular data. It has to be noted, however, that the monophyly of *L. viridescens viridior* according to barcode data, though recovered, is only poorly supported (bootstrap value of 49%). On the other hand, a significant genetic divergence was observed within *C. brunnea*; the specimen from La Paz province in Bolivia is 1.5% distant from specimens collected further south in the Chuquisaca province of the same country and in the Jujuy province of Argentina. Early investigations of this particular case suggest it is likely to represent another case of cryptic diversity, but addressing it will require further analysis of other populations of *C. brunnea*, as well as a thorough assessment of the identity of the type of this species and the elucidation of the status of a described subspecies, *C. brunnea interrupta* (Bouvier 1930), known to occur at lower elevations in Eastern Bolivia (Lemaire 2002). Calculations of genetic distances between and within species (Tables 2 and 3) also unequivocally support the distinction of species within these groups. The amount of differences accumulated in *C. speciosa* (Table 3 and Fig. 2) is remarkable and contrasts with the highly conserved morphology of that group of species.

As the specimens of *Leucanella* and *Cerodirphia* collected in the region of Muzo unambiguously form distinct lineages according to morphology and genetic data, we propose below the formal descriptions of two new species. References to records in the BOLD database are given in the following format: SampleID/ProcessID.

Leucanella bonillensis Decaëns and Rougerie, *new species*

Figs. 3–4 and 7–9

Type material. Holotype, male: Colombia, Boyacá department, Municipio de Quipama, Vereda Caviche, alt. 1500 m. a.s.l., 1–3.xii.2002, attracted to UV light, D. Bonilla and G. Lecourt leg.; genital prep. TD#171; barcode sequence BC-Dec0005/SATWA037-06. Deposited in the INCN.

Paratypes: 2 males, same data as the holotype; one with a barcode sequence available (BC-Dec0006/SATWA038-06). Deposited in the collections of the authors.

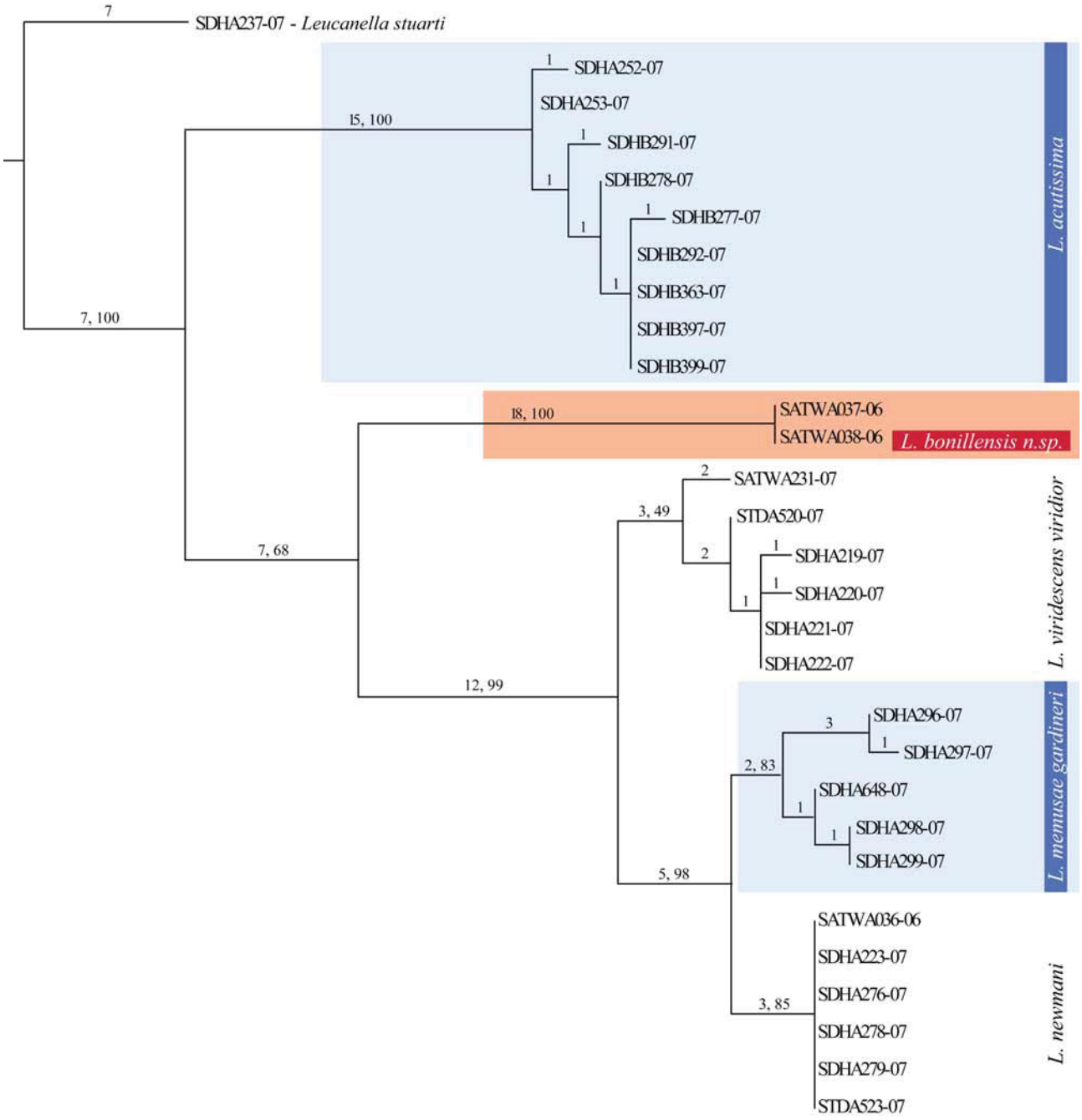


FIGURE 1. Single most parsimonious tree (L=98, CI=0.84, RI=0.96) obtained from the phylogenetic analysis of the DNA barcode sequences for the specimens of the genus *Leucanella*. Each specimen is identified by its ProcessID code (see Table 1). Branch length is proportional to the number of substitutions, and values above branches are the number of inferred changes (FAST optimization) and bootstrap supports, respectively.

Diagnosis. Based on its small size and the sharp apex of the forewings, *Leucanella bonillensis* n. sp. is most closely related to *Leucanella acutissima* and *Leucanella newmani* (Figs. 5, 6). It can be distinguished from these species by some consistent characters which are summarized in Table 4. Externally, *L. bonillensis* is slightly larger than both relatives, has less elongated and falcate forewings, and possesses a larger eyespot on the hindwing (Figs. 3, 5-6). Other diagnostic characters are the larger peri-ocellar yellow ring, compared to the narrow one that characterises both *L. acutissima* and *L. newmani* (Lemaire 2002), and the faint trace of pupil and lines on the ventral side of the hindwings (Figs. 4–6). *L. bonillensis* and *L. acutissima* also are separated clearly from *L. newmani* by the more pre-apical position of the forewing postmedial line (Figs. 3, 5-6).

Although genitalia show little variation within the genus (see discussion), we found characters that may help to distinguish *L. bonillensis* from *L. acutissima*. From the few specimens available for dissection (2 specimens of each species) and according to description of Lemaire (2002), we observed a longer bulbus ejaculatorius in *L. bonillensis* than in *L. acutissima* (Figs. 9, 12), and a greater development of the costal lobe of the valves (Figs. 7, 10). However, observations on a larger number of specimens will be necessary to assess the reliability of these genitalia features. On the other hand, we did not find any significant difference in male genitalia morphology between the new species and *L. newmani* (Figs. 7–9, 13–15).

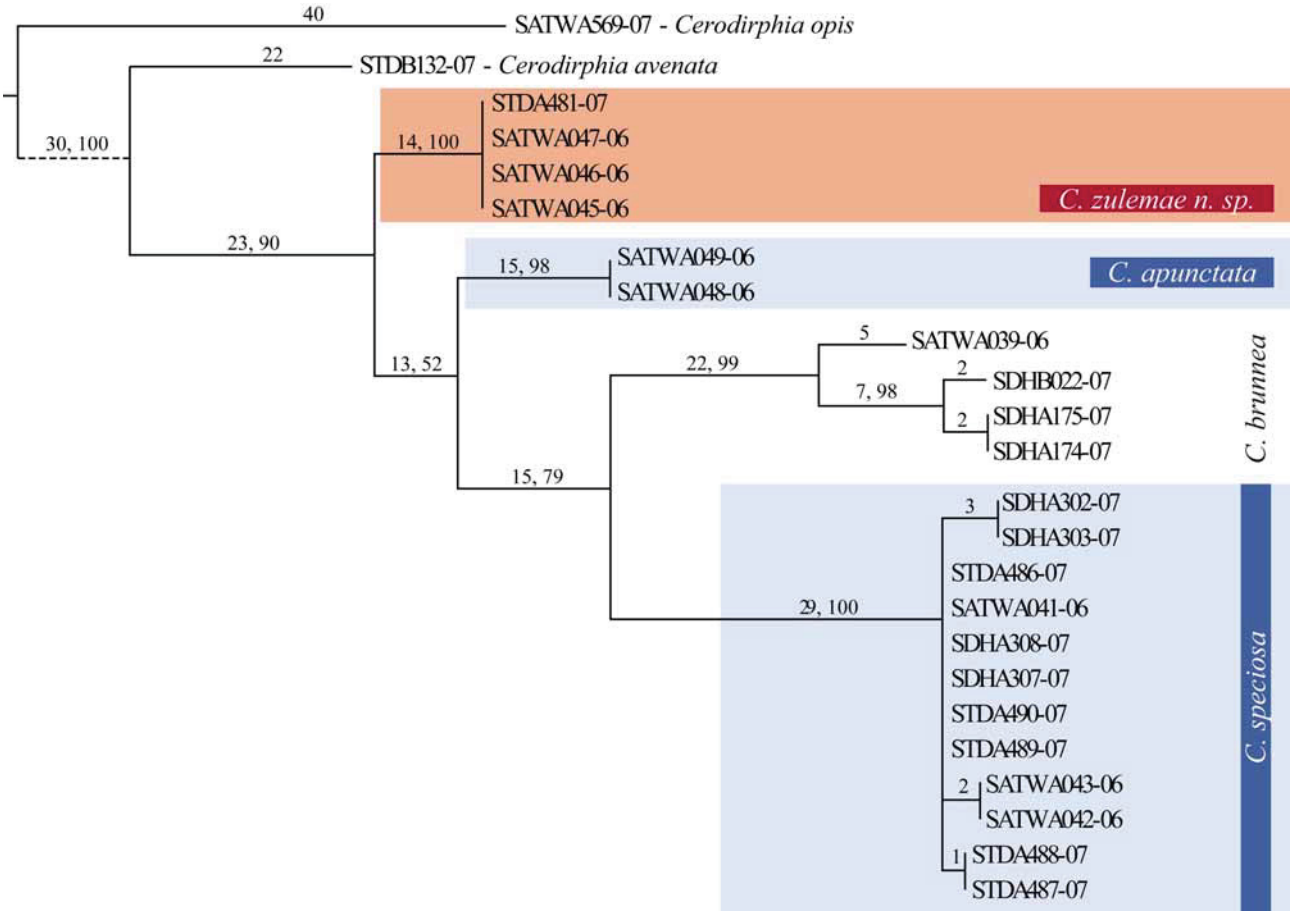


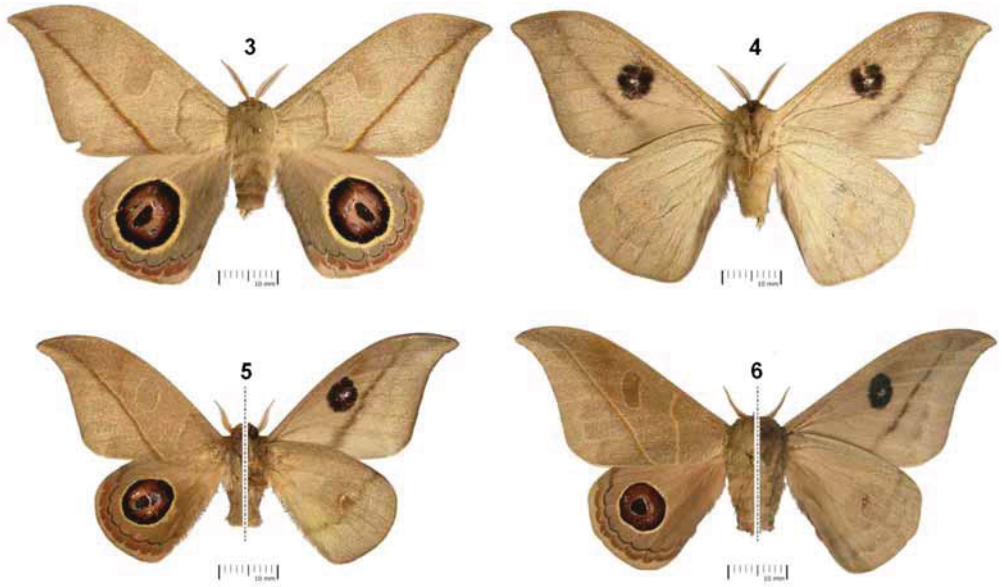
FIGURE 2. Strict consensus of the 9 most parsimonious trees (L=244, CI=75, RI=89) obtained from the phylogenetic analysis of the DNA barcode sequences for the specimens of the genus *Cerodirphia*. Each specimen is identified by its ProcessID code (see Table 1). Branch length is proportional to the number of substitutions, and values above branches are the number of inferred changes (FAST optimization) and bootstrap supports, respectively.

TABLE 2. Mean Kimura 2-parameter distances (%) for barcode DNA sequences calculated within (shaded cells) and between each of the species of the genus *Leucanella* included in our dataset.

	L. acutissima	*L. memusae gardineri*	*L. newmani*	*L. bonillensis* **n. sp.**	*L. viridescens* viridior
L. acutissima	0. 3				
L. memusae gardineri	6.4	0. 5			
L. newmani	6.8	1.2	0		
L. bonillensis **n. sp.**	6.6	6.3	6	0	
L. viridescens viridior	6.4	2.1	1.9	5.6	0.4

TABLE 3. Mean Kimura 2-parameter distances (%) for barcode DNA sequences calculated within (shaded cells) and between each of the species of the genus *Cerodirphia* included in our dataset

	C. apunctata	*C. avenata*	*C. brunnea*	*C. zulemae* n. sp.	*C. speciosa*
C. apunctata	0				
C. avenata	11.7	-			
C. brunnea	9.1	12.4	1.5		
C. zulemae n. sp.	6.7	9.3	8.6	0	
C. speciosa	10.8	16.3	10.7	12.5	0.3



FIGURES 3–6. Wing patterns of *Leucanella bonillensis* n. sp. and closely related species. 3. Male Holotype, dorsal view; 4. Idem, ventral view; 5. Male of *Leucanella acutissima*, dorsal (left) and ventral (right) views; 6. Male of *Leucanella newmani* dorsal (left) and ventral (right) views.

Description. Male (Figs. 3–4). Wingspan: 79–80 mm. *Head*: Olive to brownish grey, labial palpi of the same colour, antennae dull yellow. *Thorax*: Ventral side olive to brownish grey; legs slightly darker. Forewing length from base to apex 40 mm, elongated and falcate, with straight margin and sharp apex; above ground colour greyish; lines dark brown, the antemedial slightly lighter than the postmedial, both bordered by a line of orange scales on their facing edges, the antemedial convex, the postmedial straight and reaching the costa close to the apex; large and rectangular disco-cellular mark, slightly darker than the surrounding wing surface and externally bordered with a thin line of light scales. Hindwing with baso–medial area grey lighter near the costal margin; median area marked by a large eyespot, its iris large, red brown, with a multiple black pupil suffused with white scales, surrounded by a large and black peri-ocellar ring and an outer narrower light yellow ring; postmedial line wavy, black, followed by a narrow strip of grey scales; postmedial area reddish brown; marginal band grey. Ventral side greyish; forewing marked with a black postmedial line and a large and rounded black discal spot, marked in its centre by a large white point; hindwing uniformly greyish and devoid of remnant pupil. *Abdomen*: Ventral side olive to brownish grey; dorsal side slightly darker.

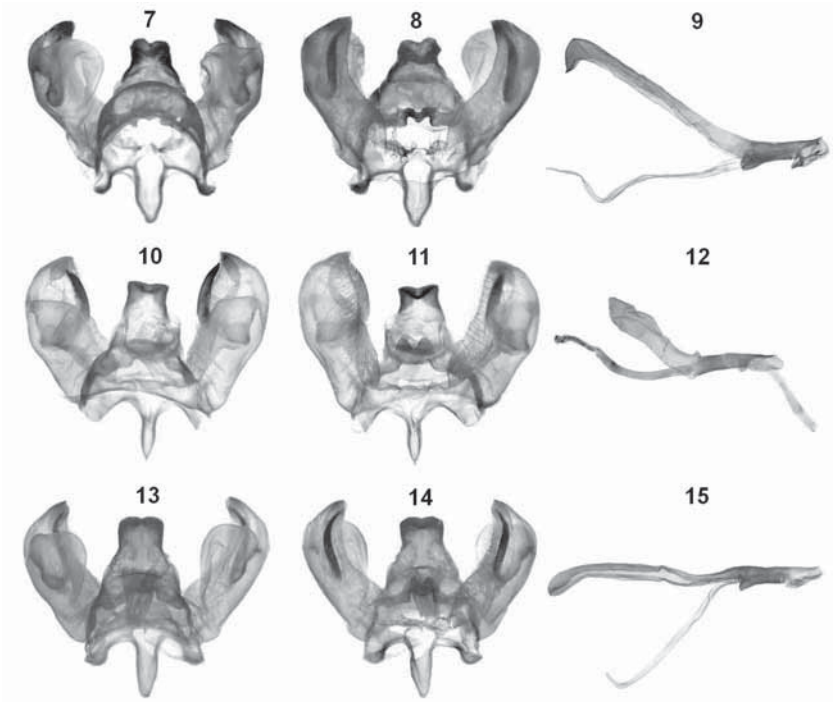
Male genitalia (Figs. 7–9). Uncus (Figs. 7–8) simple, broad and flat; its posterior part downcurved and apically rounded, bearing short dorsal setae. Transversal structure below the anal tube representing the merged gnathos and transtilla; lateral arms of the gnathos rudimentary, barely distinguishable. Median plate bilobed and moderately sclerotized. Valves short, somewhat pointed, with a distinct basal lobe arising from

the costa; this lobe (Fig. 7) weakly sclerotized, large, rounded and curved toward the median axis. Inner spine (Fig. 8) slightly curved, half the valve in length, heavily sclerotized and lodging itself along the valve's curve. Juxta membranous, possibly reduced to a pair of small setose lobes (Fig. 8) merged with the posterior margin of the vinculum; the latter laterally produced. Saccus short, straight and moderately pointed. Phallus (Fig. 9) composed of a very long, straight and apically crosier-shaped bulbus ejaculatorius and of a short (ca. one third the length of the bulbus ejaculatorius) aedeagus, the dorsal tip of which is pointed; vesica short, lacking cornuti.

Female. Unknown.

Distribution and biology. *L. bonillensis* is known only from the type locality. The list of the species collected at this locality highlights the influence of different biogeographical regions on the composition of the saturniid fauna (Decaëns et al. 2007). Hence, it is reasonable to assume that *L. bonillensis* is restricted to the Oriental Andean region, as it has never been observed in any locality of the Occidental or Amazonian areas. This point requires more collecting data to be confirmed. The immature stages and food plants are unknown.

Etymology. This species is dedicated to our friend and colleague Diego Bonilla, who collected this new species for the first time, in recognition of his collecting efforts in Colombia over the past 8 years.



FIGURES 7–15. Genitalia patterns of *Leucanella bonillensis* **n. sp.** and closely related species. 7. Holotype genitalia, dorsal view (genitalia prep. TD#171; 8. Idem, ventral view; 9. Idem, edeagus; 10. Genitalia of *Leucanella acutissima*, dorsal view (genitalia prep. TD#176); 11. Idem, ventral view; 12. Idem, edeagus; 13. Genitalia of *Leucanella newmani*, dorsal view (genitalia prep. TD#169); 14. Idem, ventral view; 15. Idem, edeagus.

Cerodirphia zulemae Decaëns and Rougerie, *new species*

Figs. 16–19, 24, 28, 32–36 and 52–54.

Type material. Holotype, male: Colombia, Boyacá department, Municipio de Quipama, Vereda Caviche, alt. 1500 m. a.s.l., 1–3.xii.2002; attracted to UV light, D. Bonilla and G. Lecourt leg.; genital prep. TD#141; barcode sequence BC-Dec0010/SATWA047-06. Deposited in INCN.

Paratypes (Figs. 17–19): 4 males (1 in collection of R. Rougerie, 2 in collection of T. Decaëns, 1 in

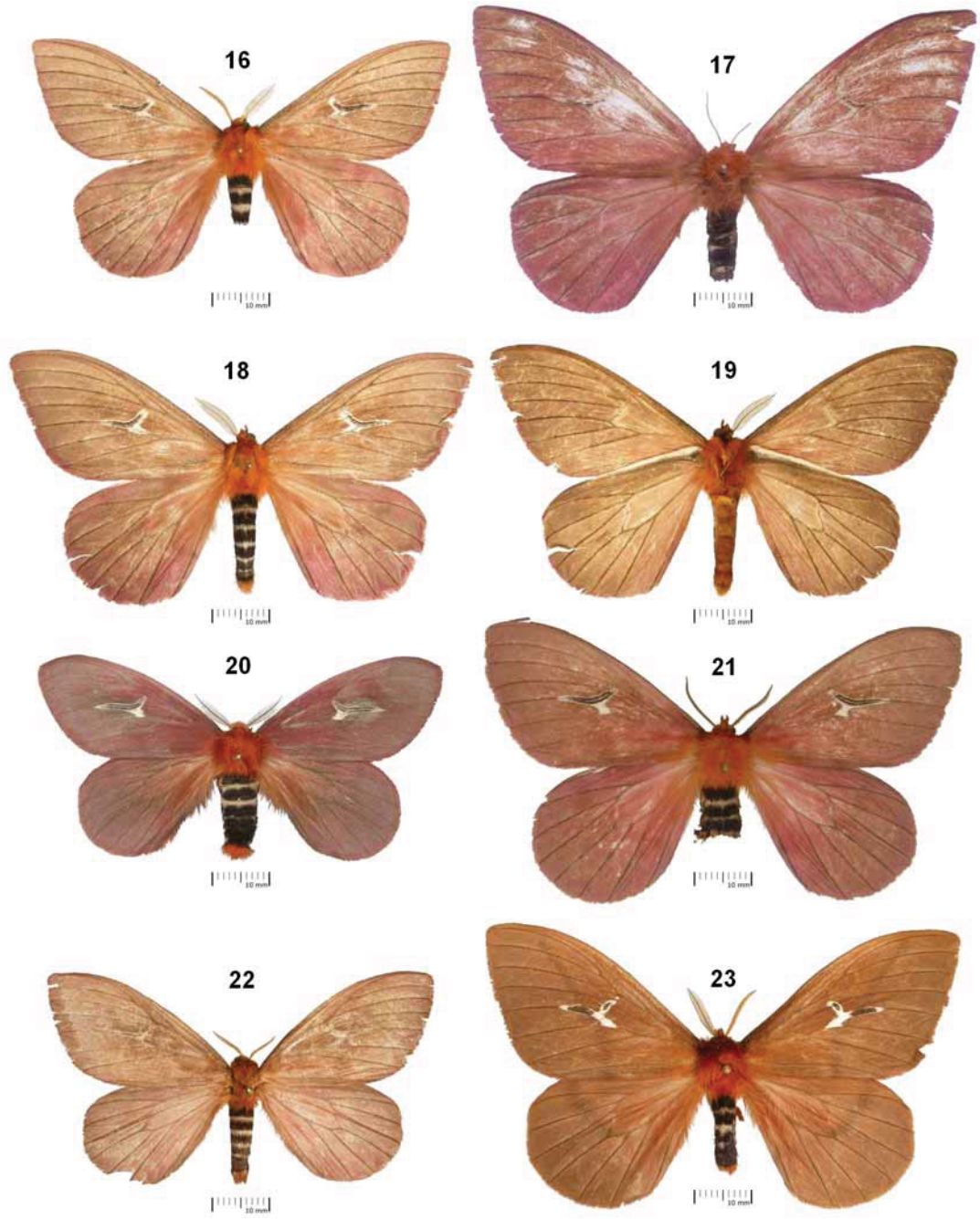
MNHN) and 2 female (one designated as allotype and deposited in INCN, the other in the collection of T. Decaëns), same data as the holotype; 3 males have barcode sequences attached on BOLD: BC-Dec0008/SATWA045-06, BC-Dec0009/SATWA046-06, BD-Dec0491/STDA481-07.

TABLE 4. Diagnostic characters to distinguish *L. bonillensis* **n. sp.** from closely related species. FW = forewings; HW = hindwings (traits in this table are relative, not absolute)..

	L. bonillensis n.sp. (Figs. 3–4, 7–9)	*L. acutissima* (Figs. 5, 10–12)	*L. newmani* (Figs. 6, 13–15)
FW shape	Less elongated	More elongated	More elongated
FW postmedial line	Straight and sub-apical	Curved and sub-apical	Curved and pre-apical
FW ante- and post-medial lines	Large, dark, bordered with a narrow yellow strip	Large, dark, with narrow yellow strip	Thin, with larger yellow strip
HW eyespot	Large diameter	Small diameter	Small diameter
HW peri-ocellar ring	Large	Narrow	Narrow
HW verso pupil	Absent	Present	Present
HW verso postmedial line	Absent	Continue	Present but interrupted
Male genitalia saccus	Large, moderately pointed	Thin, pointed	Large, moderately pointed
Male genitalia costal lobe of the valves	Large, rounded and curved toward median axis	Medium-sized, squared and curved backward	Large, rounded and curved backward
Male genitalia phallus	Bulbus ejaculatorius crosier-shaped, three times as long as the aedeagus	Bulbus ejaculatorius apically rounded, one and a half time as long as the aedeagus	Bulbus ejaculatorius apically rounded, three times as long as the aedeagus

Diagnosis. Within the genus, *Cerodirphia zulemae* n. sp. belongs to the *Cerodirphia speciosa* Cramer (1777) group (Fig. 22, 47–51), which is characterized by the vivid pink background colour of the wings and by the presence of a characteristic "Y"-shaped discal mark on the forewing. Based on the structure of the male genitalia (Figs. 32–36), *C. zulemae* appears to be closely related to *Cerodirphia brunnea* Draudt 1930 (Figs. 23, 42–46) and *Cerodirphia apunctata* Dias & Lemaire 1991 (Fig. 20–21, 37–41, Table 5) (Lemaire 2002). It can be distinguished easily from *C. brunnea* by its pink rather than brown ground colour, the more elongated hindwing, the absence of medial lines on both wings (Figs. 16, 18–19, 23), and the more rounded general shape of the male genitalia (Figs. 32–33, 42–43). It is difficult to separate *C. zulemae* from *C. apunctata* using only external characters. The somewhat sharper apex and more elongated hindwing (Figs. 16, 18–21) are in fact subtle characters that may represent intra-specific variation (Lemaire 2002). The strongest diagnostic characters thus are to be found in the male genitalia morphology (see discussion). *C. zulemae* differs mainly from *C. apunctata* by the morphology of the sclerites of the eighth abdominal segment (Figs. 24–25, 28–29) and the larger and more sclerotized cornuti of the vesica (Figs. 35–36, 40–41).

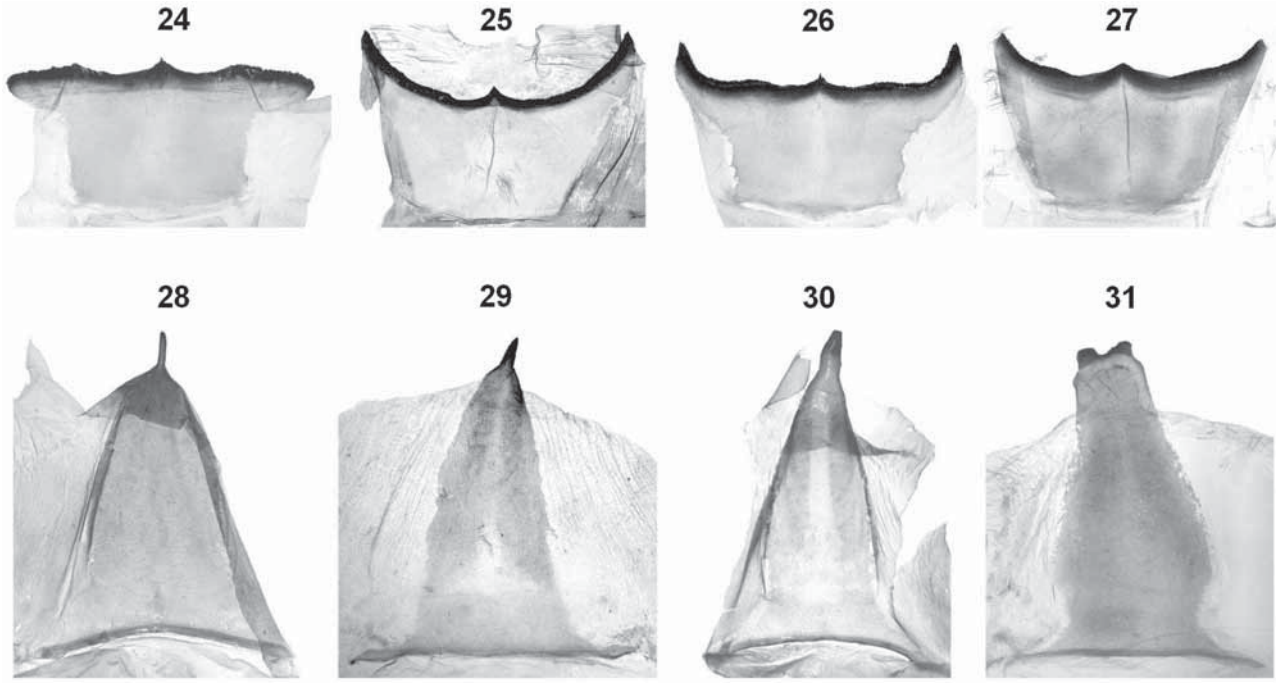
Description. Male (Figs. 16, 18–19). Wingspan: 71–80 mm. *Head*: orange, labial palpi of the same colour, antennae light orange. *Thorax*: With orange erected scales; leg vestiture a mixture of short appressed light brown scales and hair-like orange scales. Forewing length 37–42 mm, elongated with straight outer margin, rounded apex; ground colour light pink brown, with a clear pink fringe on outer margin; venation sustained by a thin web of brown scales. Classical "Y"-shaped mark extending on veins from the origin of CuA1 and bifurcating to the apical corner of the discal cell and to the first third of vein M3; this mark white, heavily sustained with dark grey scales along vein M3 and the transverse discal vein between M2 and M3. Hindwing



FIGURES 16–23. Wing patterns of *Cerodirphia zulemae* **n. sp.** and closely related species. 16. Male Holotype, dorsal vew; 17. Female Allotype, dorsal view; 18. Male paratype, dorsal view; 19. Idem, ventral view; 20. Male of *Cerodirphia apunctata*, dorsal view (French Guyana, crossroad RN2-Cacao, 25 i 1999, leg. R. Rougerie, ex larva–emerged 30 vii 1999, genitalia prep. S–RR#57); 21. Male of *C. apunctata*, dorsal view (Venezuela, Bolivar, road El Dorado – Sta Elena, km18, la Escalera, 1400m asl, 7–20 xi 1990, leg. P. Bleuzen, genitalia prep. C. Lemaire # 5718). 22. Male of *Cerodirphia speciosa*, dorsal view (French Guyana, Cayenne, i 1972, leg. J.J. de Granville, genitalia prep RR#295); 23. Male of *Cerodirphia brunnea*, dorsal view (Bolivia, La Paz, Nor Yungas, Carrasco, 1450m asl, xi 1990, leg. T. Decaëns & G. Lecourt).

with same colour pattern as forewing, with a wider extension of the areas covered by pink scales on the dorsal side; zone between vein Sc+R and the costal margin almost entirely covered with white scales on both sides of the wing; discal markings absent; baso–median dorsal area extensively covered by brownish orange hair–like scales. Ventral side same colour as dorsal with similar patterns except for the discal spot of the forewings rep-

resented by a small rectangular area of light scales. *Abdomen*: Dorsal side black with white intersegmental transversal strips; anal tuft and ventral side orange. Posterior margin of eighth sternum (Fig. 24) enlarged and highly sclerotized, minutely dentate on its external quarter and showing a small smooth concavity on each side of a short median spine; eighth tergum (Fig. 28) triangular, its posterior margin tapering to a small and thin process.

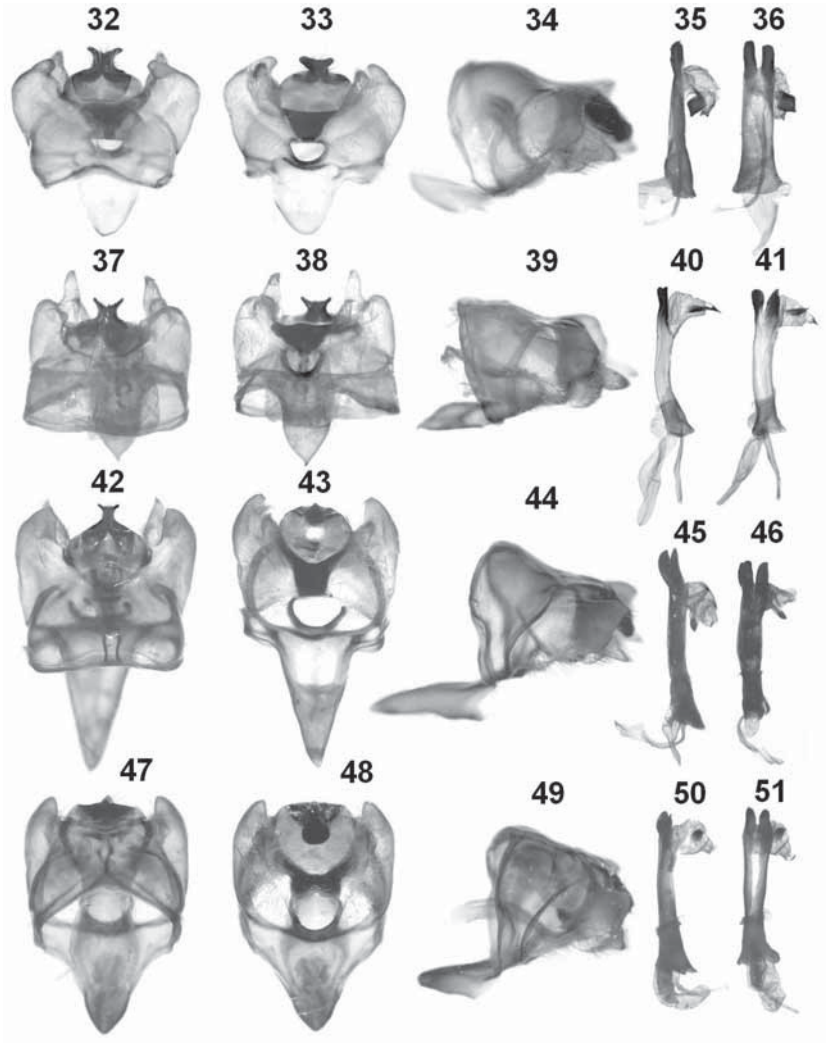


FIGURES 24–31. Genitalia patterns of *Cerodirphia zulemae* **n. sp.** and closely related species. 24. 7th tergite of the Holotype of *C. zulemae*, dorsal view (genitalia prep. TD#141); 25. Same structure, *Cerodirphia apunctata* (genitalia prep. S–RR#57); 26. Same structure, *Cerodirphia brunnea* (genitalia prep. TD#174); 27. Same structure, *Cerodirphia speciosa* (genitalia prep. RR#295); 28. 7th sternite of the Holotype of *C. zulemae*, ventral view; 29. Same structure, *C. apunctata*; 30. Same structure, *C. brunnea*; 31. Same structure, *C. speciosa*.

Male genitalia (Figs. 32–36). Uncus (Figs. 32–34) narrow, its posterior half setose, downcurved and highly sclerotized; apical part narrow then widening as a small plate with a curving rim. Gnathos present and merged with the transtilla, the median plate of which is broad, heavily sclerotized and anteriorly connected to the juxta (Fig. 33); its lateral arms merged basally with the valvae, highly setose, oriented backward and resembling small valvae. Valva short and thick (Fig. 34), produced as small tapering lobe at the apex; the base merged with the juxta (Fig. 33), the latter forming a sclerotized ring fused with the surrounding structures. Saccus short, anterior margin rounded. Aedeagus (Figs. 35–36) short, posterior part longitudinally divided in two highly sclerotized parts; caecum penis enlarged. Vesica evaginating ventrally at the longitudinal division of aedeagus, bearing two cornuti: a large and highly sclerotized square tooth and a very small apical spike.

Female (Fig. 17). Wingspan: 98 mm. Very similar to male but larger. *Head*: Dark yellow–orange, labial palpi concolourour with head, antennae light orange. *Thorax*: Orange brown; legs light orange. Forewing length 55 mm, elongate, with slightly convex border, rounded apex; above ground colour pink, scattered with dark orange brown scales, the pink colour being more vivid near the margins; venation marked with brown scales; discal markings as described for the male; proximal area covered with pink–orange hair–like scales near the anal border. Hindwing with same colour pattern but devoid of discal mark and with a wider extension of the pink areas. Ventral side with similar colour patterns as the dorsal one; forewing discal mark represented by a grey strip bordered of lighter scales; hindwings with a rectangular discal spot of light scales; area delimited by Sc+R and the costal margin dark brown externally bordered with a fringe of white hair–like scales.

Abdomen: Dorsal side black with white intersegmental transversal strips; anal tuft and ventral side dark orange brown.



FIGURES 32–51. Male genitalia patterns of *Cerodirphia zulemae* **n. sp.** and closely related species. 32. Genitalia of the Holotype of *C. zulemae*, dorsal view (genitalia prep. TD#141); 33. Idem, ventral view; 34. Idem, lateral view; 35. Idem, lateral view of the edeagus; 36. idem, dorsal view of the edeagus; 37. Genitalia of *Cerodirphia apunctata*, dorsal view (genitalia prep. S–RR#57); 38. Idem, ventral view; 39. Idem, lateral view; 40. Idem, lateral view of the edeagus; 41. idem, dorsal view of the edeagus; 42. Genitalia of *Cerodirphia brunnea*, dorsal view (genitalia prep. TD#174); 43. Idem, ventral view; 44. Idem, lateral view; 45. Idem, lateral view of the edeagus; 46. idem, dorsal view of the edeagus; 47. Genitalia of *Cerodirphia speciosa*, dorsal view (genitalia prep. RR#295); 48. Idem, ventral view; 49. Idem, lateral view; 50. Idem, lateral view of the edeagus; 51. idem, dorsal view of the edeagus.

Female genitalia (Fig. 52–54). Papillae anales setose; anterior and posterior apophyses subequal in length. Vaginal plate laterally connected to the eighth tergum, broad, with merged lamella postvaginalis; the latter (Fig. 53) composed of a pair of small sclerotized lobes and a median shield-like (Lemaire 2002: 750) structure bearing a small triangular notch. Tergum eight medially interrupted. Ostium bursae barely visible. Ductus bursae short, broad and heavily sclerotized. Corpus bursae rounded, its surface smooth.

Distribution and biology. This species is known only from the type locality at an elevation between 1500 and 2000 m a.s.l. As for *L. bonillensis*, its exact relationship with the three biogeographical regions that contribute to the Saturniidae fauna of the mountains of Muzo (Decaëns *et al.* 2007) will require more data to be defined precisely. The presence of both species on the other side of the Oriental Cordillera (which goes down

to the Eastern Plains) also requires investigation. For example, a female of *Cerodirphia* from the Amazonian Piedmont of the Caqueta department (Racheli & Vinciguerra 2005) should be compared carefully to *C. zulemae*. Immature stages and biology are unknown

Etymology. This species is dedicated to the wife of the senior author, acknowledging her patience and collaboration during all the collecting trips made by D. Bonilla, L. D. Ramirez and T. Decaëns in Colombia.

TABLE 5. Diagnostic characters to distinguish *C. zulemae* **n. sp.** from closely related species. FW = forewings.

	C. zulemae **n. sp.** (Figs. 16–19, 24, 28, 32–36)	*C. apunctata* (Figs. 20–21, 25, 29, 37–41)	*C. speciosa* (Figs. 22, 27, 31, 47–51)	*C. brunnea* (Figs. 23, 26, 30, 42–46)
FW shape	Elongated, acute apex	Less elongated, rounded apex	Elongated, acute apex	Less elongated, acute apex
Ground colour	Vivid pink	Vivid pink to brown–pink	Vivid pink	Brown–pink
Lines	Absent	Absent	Absent	Medial lines present
Male genitalia overall shape	Rounded	Rounded	Rounded	More elongated
Male genitalia uncus/valves relative development	Uncus distinctly developed posteriorly to a greater extent than the valves	Posterior development of uncus and valves subequal	Same as for *C. apunctata*	Same as for *C. apunctata*
Male genitalia cornuti	Two: a large square tooth and a small apical spike	Two: a medium-sized pointed tooth and an apical spike	A single medium-sized tooth	A single medium-sized tooth
Male genitalia saccus	Apex rounded, moderate development	Apex pointed, moderate development	Apex slightly pointed, moderate development	Triangular, strong development
Male abdomen eight sternum	Posterior margin roughly straight	Posterior margin clearly biconcave	Posterior margin clearly biconcave	Posterior margin clearly biconcave
Male abdomen eighth tergum	Thin sclerotized posterior process	Large sclerotized posterior process	Large sclerotized posterior process	Bifid sclerotized posterior process

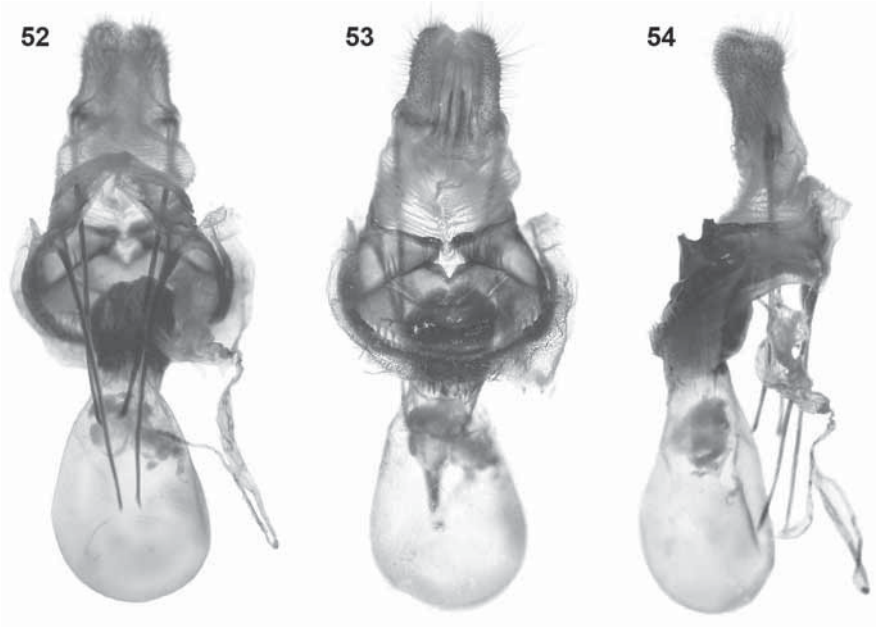
Discussion

The morphological evidence justifying the two new species described in this paper strongly differ, mainly because of significant differences in the variability of the genera they belong to. While the characters that distinguish *Leucanella bonillensis* from closely related species are mainly wing patterns, specific features for *Cerodirphia zulemae* are largely male genitalia. Genitalia have been reported to provide few species-level characters within *Leucanella* (Lemaire 1973; Lemaire 2002). As a result, most species descriptions have been based on wing characters which are similar to those we used for *L. bonillensis*. As an example, Lemaire (1973) considered the small wing size, the elongated shape, and acute apex of the hindwings, as well as the thin yellow periocellar ring on the forewings as important features characterising the species group of *L. acutissima*. On the other hand, he gave few if any significant genitalia characters to distinguish *L. acutissima* from *L. newmani*. If *L. bonillensis* clearly differs from these two species by its larger periocellar ring, it conforms to the group characteristics with regards to the other characters, and the male genitalia appear too conserved to constitute a reliable diagnostic feature.

Conversely, within the genus *Cerodirphia,* the *C. speciosa* species-group is characterised by the constancy

of the wing patterns but marked differentiation in genitalia. Several species have been described recently in this genus on the basis of characters such as the development and size of the cornuti and the shape of the sclerotized structures on the eighth abdominal segment. This was, for example, the case for *C. apunctata* (Dias & Lemaire 1991) as well as species belonging to other species groups within the genus (e.g., Rougerie and Herbin 2004; but see Lemaire 1973, 2002 for a complete presentation).

In this context, we emphasize the value of combining morphological observations in such difficult groups with the additional independent dataset of DNA barcode sequences. We conclude that the ongoing assemblage of a comprehensive DNA barcode library for all members of the Saturniidae, including specimens from various geographical origins across the distribution of each species, represents a promising powerful resource for taxonomists to efficiently and rapidly explore biodiversity by screening genetic divergences for evidence of cryptic species or other taxonomic issues.



FIGURES 52–54. Female genitalia patterns of *Cerodirphia zulemae* **n. sp.** 52. Genitalia of the Allotype, dorsal view (genitalia prep. TD#184); 53. Idem, ventral view; 54. Idem, lateral view.

Acknowledgments

We are particularly grateful to Diego Bonilla, Luz Dary Ramirez, and Gilbert Lecourt who collected the first specimens of these two new species. Alex Smith, Paul D.N. Hebert, and Kirby Wolfe made useful comments and corrections to the manuscript; the later also provided two specimens of *L. acutissima* for comparisons. Joel Minet, curator of saturniids at MNHN, kindly granted access to the collections and specimens of this institution. Daniel Herbin also kindly provided sequences of specimens from different species deposited his own collection. Barcode analyses were carried out through grants from NSERC and Genome Canada through the Ontario Genomics Institute.

Literature cited

Amarillo-S., A.R. (2000) Polillas Satúrniidas (Lepidoptera: Saturniidae) de Colombia. *Biota Colombiana* (Bogotá), 1(2), 177–186.

Amarillo, A.R. & Wolfe, K.L. (1997) Descripción del ciclo de vida y ampliación de ámbito de *Rothschildia zacateca*

(Lepidoptera: Saturniidae). *Tropical Lepidoptera* (Gainesville), 8(2), 71–74.
Decaëns, T. (2003) *Dirphia carimaguensis*, a new Hemileucinae from the Eastern Plains of Colombia (Lepidoptera: Saturniidae). *Galathea* (Nurnberg), supplement 15, 13–21.
Decaëns, T., Bonilla, D. & Ramirez, L.D. (2003a) A new *Gamelia* from the Pacific Region of Colombia (Lepidoptera: Saturniidae, Hemileucinae). *Galathea* (Nurnberg), supplement 16, 173–180.
Decaëns, T., Bonilla, D. & Ramirez, L.D. (2003b) *Meroleuca (Meroleucoides) diazmaurini*, a new Saturniidae from Central Colombia (Lepidoptera: Saturniidae, Hemileucinae). *Galathea* (Nurnberg), supplement 15, 47–53.
Decaëns T., Bonilla D., Ramirez L.D., Amarillo A., Wolfe K., Brosch U. & Naumann S. (2003c) Diversidad de Saturniidae (Lepidoptera) en la selva andina de San José del Palmar (Alto Chocó, Colombia). *Boletín Científico del Museo de Historia Natural* (Manizales), 7, 57–75.
Decaëns, T., Bonilla, D. & Wolfe, K. (2003d) A new *Hylesia* (Lepidoptera: Saturniidae, Hemileucinae) from the National Park of Iguaque (Eastern Cordillera, Colombia). *Nachriten des Entomologischen Vereins Apollo* (Frankfurt am Main), 24(1/2), 39–46.
Decaëns, T., Rougerie, R., Bonilla, D., Ramirez, L.D. (2007) Contribution to the knowledge of the Saturniidae fauna of Muzo (Boyaca, Colombia), with the redescription of *Copaxa apollinairei* Lemaire. *Nachrichten des Entomologischen Vereins Apollo* (Frankfurt am Main), 28(1/2), 69–75.
Dias, M.M. & Lemaire, C. (1991) Description d'un *Cerodirphia* inédit (Lepidoptera Saturniidae Hemileucinae). *Lambillionea* (Tervuren), 91, 272–277.
Goloboff, P.A. (1999) NONA. Program and Documentation, Version 2.0. Published by the author.
Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L., Mackie, P. & Hebert, P.D.N. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London (Series B)*, 360, 1959–1967.
Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Science USA*, 103, 968–971.
Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London (Series B)*, 270, 313–321.
Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator. Proceedings of the National Academy of Science USA*, 101, 14812–14817.
Ivanova, N., deWaard, J. & Hebert, P. (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes*, 6, 998–1002.
Jordan K. (1924) On the Saturnoidean families Oxytenidae and Cercophanidae. *Novitates Zoologicae*, 31, 135–193.
Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
Lemaire, C. 1973. *Révision du genre Automeris Hübner et des genres voisins. Biogéographie, éthologie, morphologie, taxonomie (Lép. Attacidae)*. Mémoires du Muséum National d'Histoire Naturelle, série A, Zoologie, LXXIX, 208 pp.
Lemaire, C. (1978) *Les Attacidae Américains – The Attacidae of Americas. Part 1 – Attacinae*, C. Lemaire Editions, Neuilly sur Seine, 238 pp.
Lemaire, C. (1980) *Les Attacidae Américains – The Attacidae of Americas. Part 2 – Arsenurinae*, C. Lemaire Editions, Neuilly sur Seine, 199 pp.
Lemaire, C. (1988) *Les Saturniidae Américains – The Saturniidae of Americas – Los Saturniidae Americanos. Part 3 – Ceratocampinae*, Museao Nacional de Costa Rica, San José, 480 pp.
Lemaire, C. (1996) Saturniidae. *In:* Heppner, J.B. (Ed) *Atlas of Neotropical Lepidoptera. Checklist: Part 4B. Drepanioidea – Bombycoidea – Sphingoidea*. Association for Tropical Lepidoptera – Scientific Publishers, Gainesville, pp. 28–49.
Lemaire, C. (2002) *Les Attacidae Américains – The Attacidae of Americas – Los Saturniidae Americanos. Part 4 – Hemileucinae*, Goecke & Evers, Keltern, 1388 pp. + 143 pp. of colour plates without pagination.
Lemaire, C. & Amarillo, A. (1992) Une nouvelle espèce du genre *Automeris* de Colombie Orientale (Lepidoptera : Saturniidae). *Tropical Lepidoptera* (Gainesville), 3(2), 153–154.
Minet, J. (1994) The Bombycoidea: phylogeny and higher classification (Lepidoptera : Glossata). *Entomologica Scandinavica*, 25, 63–88.
Nixon, K.C. (2002) *WinClada, Version 1.00.08.* Published by the author, Ithaca: New York.
Racheli, L. & Vinciguerra, R. (2005) Notes on some Saturniidae from Albania (Caqueta department) including new records for Colombia (Lepidoptera: Saturniidae). *Shilap Revista de Lepidopterologia* (Madrid), 33 (130), 225–229.
Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System. *Molecular Ecology Notes*, 7(3), 355–364.

Regier, J.C., Cook, C.P., Mitter, C., & Hussey, A. (2008) A phylogenetic study of the 'bombycoid complex' (Lepidoptera) using five protein-coding nuclear genes, with comments on the problem of macrolepidopteran phylogeny. *Systematic Entomology*, 33, 175–189.

Rougerie, R. & Herbin, D. (2004) A new *Cerodirphia* from Peru (Lepidoptera: Saturniidae, Hemileucinae). *Nachrichten des Entomologischen Vereins Apollo* (Frankfurt am Main), 25 (3), 145–147.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.

Wolfe, K.L., Bonilla, D., Ramirez, L.D. & Decaëns, T. (2003a) Rediscovery of *Copaxa sapatoza* and revealing of its immature stages (Lepidoptera: Saturniidae, Saturniinae). *Nachriten des Entomologischen Vereins Apollo* (Frankfurt am Main), 24(3), 143–146.

Wolfe, K.L., Lemaire, C., Amarillo-S., A. & Conlan, C.A. (2003b) A contribution to the systematics of the *Copaxa semioculata* species–group (Saturniidae), with notes on the early stages, and a description of *Copaxa lunula*, new species. *Journal of the Lepidopterists' Society* (Los Angeles), 57(1), 54–61.